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千针万线草环肽 A 的结构修正*

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摘要 从云南民间药物千针万线草（*Stellaria yunnanensis*）的根中分离到一新环肽 stellarin A。前报主要利用快速原子轰击质谱确定其结构为 cyclo(Gly-Pro-Phe-Tyr-Gly-Gly-Pro)。最近利用最新二维核磁结合快速原子轰击质谱及酶降解重新考察了其结构，修正为 cyclo(Gly-Pro-Phe-Pro-Gly-Tyr-Gly)。

关键词 千针万线草, 石竹科, 环肽, 千针万线草环肽 A

REVISION OF THE STRUCTURE OF STELLARIN A

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Abstract From the fresh roots of *Stellaria yunnanensis* Franch. a new cyclopeptide named stellarin A, was obtained and its structure was established to be cyclo(Gly-Pro-Phe-Tyr-Gly-Gly-Pro) by FAB-MS in a previous communication. Recently by a combination of 2D-NMR, FAB-MS and enzymatic degradation, its structure was re-examined and revised to cyclo(Gly-Pro-Phe-Pro-Gly-Tyr-Gly).

Key words *Stellaria yunnanensis*, Caryophyllaceae, Cyclopeptides, Stellarin A

INTRODUCTION

As part of program of our searching for biologically active cyclopeptides from plant resources[1—4], we

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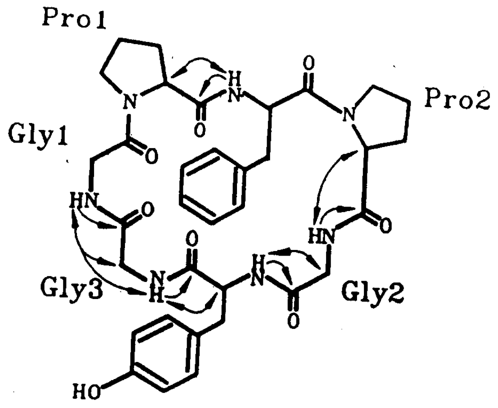
recently have investigated the constituents of the fresh roots of *Stellaria yunnanensis* Franch used as a lung tonic in Yunnan, China[5] and obtained a new cyclopeptide named stellarin A. The structure was established by means of FAB–MS in a previous communication[6]. Recently, the structure was re–examinedand revised to cyclo(Gly–Pro–Phe– Pro–Gly–Tyr–Gly) from the structure, cyclo(Gly–Pro–Phe–Tyr–Gly–Gly–Pro), reported in the previous communication. In this paper, the structural elucidation was reported in detail.



Scheme 1: Selected HMBC and NOESY's for stellarin A in DMSO–d_6

A methanolic extract of the fresh roots of *S. yunnanensis* was suspended in water, extracted with petroleum–benzene, EtOAc and n–BuOH, respectively. EtOAc extract was concentrated to afford a residue(37g). The residue was submitted to column chromatography on silica gel, eluted with $CHCl_3$ containing increasing proportion of MeOH to give stellarin A.

RESULTS AND DISCUSSION

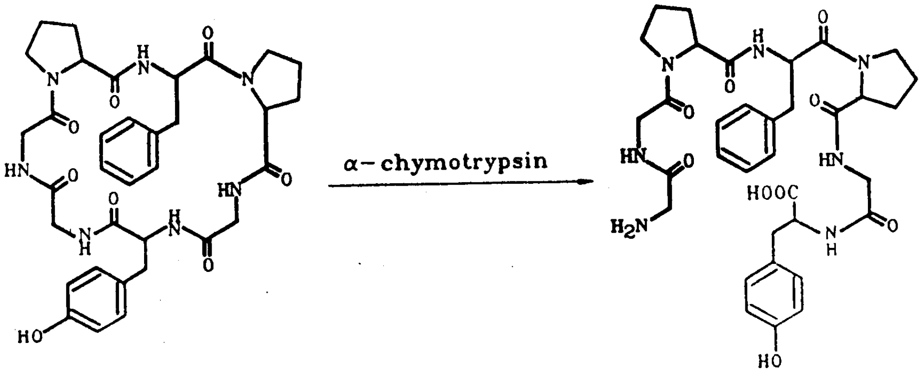
Stellarin A(1) , amorphous powder, $[\alpha]_D^{19}-11.4$(c 0.696, MeOH), is negative to ninhydrin reaction but positive to the same reaction after hydrolyzed with 6 mol / L HCl. Its molecular formula was determined to be $C_{34}H_{41}O_8N_7$ by HRFAB–MS[$(M+1)^+$ at m / z 676.315, calc. 676.309]. The IR spectrum exhibited intense N–H and C=O absorptions at $\nu_{max}=3300$ and 1650 cm^{-1}, respectively. The ^{13}C NMR spectrum showed the presence of seven amide carbonyls(168.21, 168.45, 170.43, 170.23, 172.25, 170.33, 170.43). These facts indicated that 1 is a cyclopeptide.

Analyses of the NMR spectra were begun with identification of spin systems of the different amino acid residues. The amino acid residues were readily identified by means of 2D NMR techniques. The 600MHz ^1H NMR spectrum indicated that one conformation strongly dominated in DMSO–d_d. The ^1H NMR spectrum clearly showed the presence of five amide hydrogen atoms. By following the spin systems of these hydrogen atoms using DQF–COSY and TOCSY, these amino acid residues were found to be three glycine, phenylalanine and tyrosine units. Tyrosine and phenylalanine residue all contain AMXY spin system with comparable chemical shift values and coupling constants, and therefore exhibited a similar COSY spectrum, but differentiation between tyrosine and phenylalanine unit can be achieved by means of HMBC. The remaining NMR signals were revealed to consist of two independent spin systems of the type ($X-CH-CH_2-CH_2-CH_2-X$) typical of proline[7]by a combination of DQF–COSY, TOCSY, HMQC and HMBC. The seven amino acid residues accounted for the mol wt. observed in FAB–MS spectrum. Amino acid analysis of the hydrolysate prepared with 6 mol / L HCl also confirmed the presence of the amino acid units established by NMR techniques.

Evidence for the sequence of the amino acid residues was provided by HMBC[8], NOESY[9]and

FAB-MS[10]. As shown in Scheme 1, the HMBC and NOESY correlations indicated the presence of two peptide fragments(-Pro_2-Gly_2-Tyr-Gly_3-Gly_1-, -Pro_1- Phe-). The fragments at m/z 211, 398 observed in FAB-MS implied the presence of two peptide fragments(-Gly-Gly-Pro-, -Pro-Phe-Pro-Gly-). The combination of these results led to the assignment of 1 as cyclo(Gly_1-Pro_1-Phe-Pro_2-Gly_2-Tyr-Gly_3).

To corroborate the proposed structure for stellarin A(1), 1 was, as shown in Scheme 2, hydrolyzed with α-Chymotrypsin, and the hydrolysate was purified by HPLC(ODS, 1/25cm, Ultrasphere) to afford a peptide(AE)[$(M+1)^+$at 694]. The peptide showed positive reaction to ninhydrin. In the manual DABITC-PITC double coupling method, the sequence of the peptide was determined to be Gly-Gly-Pro-Phe-Pro-Gly-Tyr. The results metioned above further confirmed the structure assigned to stellarin A(1) by spectroscopic method.



Scheme 2 enzymatic hydrolysis of stellarin A with α-chymotrysin

Unambiguous assignments of the ^1H, ^{13}C NMR signals of 1 were carried out by means of 2D-NMR techniques includig ^1H-^1H COSY, TOCSY, HMQC, HMBC.

EXPERIMENTAL

General. Optical rotation was measured in MeOH. UV spectrum was measured in EtOH. IR: KBr disc. ^1H, ^{13}C and 2D-NMR spectra were recorded at 600MHz in DMSO-d_6 using TMS as int. std.

Plant material. The fresh roots of *S. yunnanensis* Franch. were collected from Kunming, China in June, 1992 and identified by Prof. Z. Y. WU. A voucher specimen is deposited in the Herbarium of the Kunming Institute of Botany.

Extraction and isolation. The fresh roots of *S. yunnanensis* Franch. were extracted with MeOH under reflux. After removal of solvent by evapn. the combined extracts were suspended in H_2O, extracted with petroleum benzene, EtOAc and n-Bu-OH, respectively. After being washing with H_2O, the EtOAc extract was evapd *in vacuo* to give a residue(37g). The residue was submitted to column chromatography on silica gel, eluting with $CHCl_3$ containing increasing proportion of MeOH. Fractions were monitored by TLC. The compound was further purified by column chromatography on silica gel to afford a cyclopeptide named stellarin A(180mg, 0.02%).

Stellarin A(1), $C_{34}H_{41}O_8N_7$, amorphous powder, negative reaction to ninhydrin, $[\alpha]_D^{19}-11.4$(c 0.696, MeOH). UVλ_{max}^{EtOH}nm(logε): 203(4.56), 275(3.25), 283(3.18). IRν_{max}^{KBr}cm^{-1}: 3300, 1650, 1525, 1470. FAB–MS m / z(%): 676[$(M+1)^+$, base peak], 471, 455, 429, 387, 277, 199. HRFAB–MS: 676.309, calcd for $C_{34}H_{41}O_8N_7$, found. 676.315. ^{1}H NMR(600MHz, DMSO–d_6, TMS, δppm): Gly$_1$: 9.150(d, 3.6, H_N), 4.050(d, 14.4, $H_{\alpha1}$), 3.409(d, 14.4, $H_{\alpha2}$); Gly$_2$: 8.945(d, 4.8, H_N), 3.950(dd, 6.5, 13.2, $H_{\alpha1}$), 3.319(ca, over, $H_{\alpha2}$); Gly$_3$: 7.962(d, 7.2, H_N), 4.280(ca. over, $H_{\alpha1}$), 3.561(d, 16.2, $H_{\alpha2}$); Phe: 7.593(d, 9.6, H_N), 4.963(m, H_α), 2.990(d, 13.2, $H_{\beta1}$), 3.370(d, 13.2, $H_{\beta2}$), ArH: 7.202–7.428; Tyr: 7.662(d, 10.2, H_N), 4.462(dd, 6.6, 3.2, $H_{\alpha1}$), 2.888(6.6, 13.5 $H_{\beta1}$), 2.635(7.4, 13.5, $H_{\beta2}$), ArH: 6.579–6.952; Pro$_1$: 4.263(m, H_α), 1.751(m, $H_{\beta1}$, 1.503(m, $H_{\beta2}$), 1.519(m, $H_{\gamma1}$, 0.739(m, $H_{\gamma2}$), 4.609(t, 7.2, $H_{\delta1}$), 3.403(ca, over, $H_{\delta2}$); Pro$_2$: 4.215(dd, 7.8, 4.2, H_α), 2.161(m, $H_{\beta1}$), 1.880(m, $_{\beta2}$), 2.085(m, $H_{\gamma1}$), 1.873(m, $H_{\gamma2}$), 4.252(m, $H_{\delta1}$), 3.924(m, $H_{\delta2}$). ^{13}C NMR(600MHz, DMSO–d_6, TMS, δppm): Gly$_1$: 168.21(C=O), 41.86(C_α); Gly$_2$: 168.45(C=O), 42.60(C_α); Gly$_3$: 170.43(C=O), 41.32(C_α); Tyr: 170.33(C=O), 56.09(C_α), 38.32(C_β), 126.99(C_γ), 115.02, 155.88, 129.81; Phe: 170.43(C=O), 51.04(C_α), 36.58(C_β), 137.31(C_γ), 128.19, 129.36, 126.51. Pro$_1$: 170.23(C=O), 59.70(C_α), 29.01(C_β), 22.57(C_γ), 46.54(C_δ). Pro$_2$: 172.25(C=O), 60.69(C_α), 29.07(C_β), 24.74(C_γ), 47.98(C_δ).

Amino acid analysis of stellarin A(1) . In a routine method, amino acid analysis of the hydrolysate prepared from stellarin A(1) with 6 mol / L HCl by heating at 110℃ for 24 h in a sealed tube exhibited the presence of glycine(3 eq.), proline(2 eq.), phenylalanine(1 eq.) and tyrosine(1 eq.).

Enzymatic hydrolysis of stellarin A(1) with α–Chymotrypsin: 0.4 mg of α–Chymotrypsin was suspended in 1 ml of 4.2 mg of stellarin A(1) in 0.2 mol / L N–methylmorpholine acetate buffer (pH 8.1). After blended fully, the mixture was incubated at 38℃ until the reaction mixture showed positive reaction to ninhydrin on TLC. The reaction mixture was purified by HPLC (ODS, 1 / 25 cm, Ultrasphere), eluted with MeOH–H_2O (6:4) to afford a peptide(AE)[1.4mg, $(M+1)^+$at m / z 695, ninhydrin reaction(+)]. In the manual DABITC–PITC double coupling method, it was determined to be Gly–Gly–Pro–Phe–Pro–Gly–Tyr.

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